binding domain. The well is then extensively washed with physiologic saline buffer, after which target cells to the virus are incubated in the well to determine the level of infectious activity remaining in the well. The reduction in infectious activity, or titer, relative to the initial viral supernatant is assessed and compared to that of a similar control run (e.g. using a BSA-coated well). A significantly higher titer remaining in the Heparin-II domain containing well as compared to the control well signifies that the subject virus is suitable for use in aspects of the invention. To facilitate this screening procedure, the viral vector may contain a selectable marker gene, as discussed above.

Fragments of fibronectin for use in the invention can be of natural or synthetic origin, and can be prepared in substantial purity from naturally-occurring materials, for example as previously described by Ruoslahti et al. (1981) *J. Biol. Chem.* 256: 7277 ; Patel and Lodish (1986) *J. Cell. Biol.* 102:449; and Bernardi et al. (1987) *J. Cell. Biol.* 105:489. In this regard, reference herein to a substantially pure fibronectin or fibronectin fragments is intended to mean that they are essentially free from other proteins with which fibronectin naturally occurs. Substantially pure fibronectin or fibronectin fragments for use in the invention can also be recombinantly produced, for instance as generally described in U.S. Patent No. 5,198,423 issued March 30, 1993 to Taguchi et al. and assigned to Takara Shuzo Co., Ltd., Kyoto, Japan. In particular, the recombinant fragments identified in the Examples below as H-271, H-296, CH-

271, CH-296 and C-CS1, and methods for obtaining them, are described in detail in this '423 patent. The C274 fragment utilized in the Examples below was obtained as described in U.S. Patent No. 5,102,988. These fragments or fragments from which they can be routinely derived are available by culturing E. coli deposited at the Fermentation Research Institute of the Agency of Industrial Science and Technology, Japan as FERM P-10721 (H-296), FERM BP-2799 (C-277 bound to H-271 via methionine), FERM BP-2800 (C-277 bound to H-296 via methionine), and FERM BP-2264 (H-271), as also described in U.S. Patent No. 5,198,423. In addition, useful information as to fibronectin fragments utilizable herein or as to starting materials for such fragments may be found in Kimizuka et al., *J. Biochem.* 110, 284-291 (1991), which reports further as to the above-noted recombinant fragments; in *EMBO J.*, 4, 1755-1759 (1985), which reports the structure of the human fibronectin gene; and in *Biochemistry*, 25, 4936-4941 (1986), which reports on the Heparin-II binding domain of human fibronectin. Fibronectin fragments which contain both the CS-1 cell adhesion domain and the Heparin-II binding domain, for example as included in about a 30 or 35 kd fragment (30/35 FN) and in various recombinant fragments as reported in the Examples below, have been found to significantly enhance the efficiency of gene transfer into hematopoietic cells in work thus far, and are preferred for use in the invention. It will thus be understood that, broadly speaking, the fibronectin-related polypeptide or polypeptides utilized in the invention will provide an amino acid sequence providing the cell-binding activity of the CS-1

cell adhesion domain of fibronectin as well as an amino acid sequence of the Heparin-II binding domain of fibronectin which binds the virus. The skilled artisan will recognize that the necessary cell- and virus-binding activities can be provided both by the native amino acid sequences of these functional fibronectin domains and by amino acid sequences which differ from the native sequences yet are sufficiently similar to exhibit the cell-binding and viral-binding activities. These similar amino acid sequences will exhibit substantial sequence homology to their corresponding native sequences, and can include those in which amino acids have been deleted, substituted for and/or modified while nonetheless providing an amino acid sequence with the desired cell-binding or viral-binding characteristic.

In this regard, the pertinent biotechnological arts have advanced to a state in which the deletion, substitution, addition or other modification of amino acids in the subject functional domains can be routinely performed. The resulting amino acid sequences can then be routinely screened for the desired cell-binding or viral-binding activity. For example, viral-binding activity of mutant or modified forms of the Heparin-II-binding domain of fibronectin can be screened as generally discussed above and more specifically below in Examples 8 and 9, using virus incubation, wash, and viral titer assays to determine the retention of infectiousness compared to a control. Given the teachings provided herein, these binding assays

will represent but routine experimentation to those working in this field.

Cell-binding to modified or mutant forms of the CS-1 cell adhesion domain of fibronectin, or to other cell-binding polypeptides, can likewise be assayed using conventional procedures. For example, such procedures include those described in *Nature* 352: 438-441 (1991). Briefly, the cell-binding polypeptide is coated on plastic dishes and the cell population to be assayed is overlayed in medium for 30 minutes to 2 hours. After this incubation period, cells non-adherent to the protein are retrieved, counted and assayed for viability. Cells adherent to the polypeptide are also retrieved using trypsin or cell dissociation buffer (e.g. Gibco), counted and viability tested. In some cases, for example for hematopoietic colony forming cells, the cells are further cultured for an additional 12-14 days to ascertain the colony forming characteristics of the cells. The percentage of adherent cells is then calculated and compared to standard to a standard control such as bovine serum albumin (BSA) coated plastic dishes. Substantial binding of the target cells to the assayed polypeptide provides an indication that the polypeptide/cell combination is suitable for the invention, and the polypeptide can be coupled to the retroviral binding fragment from fibronectin to produce a construct of the invention for enhancing the infection of the target cells by the viral vector.

Pursuant to more specific aspects of the invention, the viral-binding polypeptide utilized to enhance transduction by retroviral vectors will comprise (i) a first amino acid sequence which corresponds to the Ala^{1690} - Thr^{1960} of the Heparin-II binding domain of human fibronectin, which is represented by the formula (Seq. I.D. #1):

Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Sys Pro Asp Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Sys Pro Gly Sev Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys Thr;

or a sufficiently similar amino acid sequence thereto to exhibit the ability to bind the retrovirus;

and (ii) a second amino acid sequence which corresponds to one portion of the IIICS binding domain of human fibronectin (the CS-1 cell binding domain); which is represented by the formula (Seq. I.D. #2):

Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr;

or a sufficiently similar amino acid sequence thereto to exhibit the ability to bind hematopoietic cells such as primitive progenitor and/or long term repopulating (stem) cells.

As mentioned previously, it will be understood that certain modifications and/or mutations of these native sequences are possible within the practice of the present invention, so long as the resulting amino acid sequence is sufficiently similar to the native sequence to exhibit the ability to bind the virus (in the case of the Heparin-II-binding domain) and the ability to bind the target cells (in the case of the CS-1 domain).

One aspect of the invention provides a method of somatic gene therapy which involves *in vitro* cellular therapy and subsequent transplantation of target cells into a host, also known as "engraftment" of the host with the transduced target cells. Hematopoietic or other cells can be collected from a human or other mammalian animal source using standard protocols. For example, the hematopoietic cells can be collected from bone marrow or